



Early Journal Content on JSTOR, Free to Anyone in the World

This article is one of nearly 500,000 scholarly works digitized and made freely available to everyone in the world by JSTOR.

Known as the Early Journal Content, this set of works include research articles, news, letters, and other writings published in more than 200 of the oldest leading academic journals. The works date from the mid-seventeenth to the early twentieth centuries.

We encourage people to read and share the Early Journal Content openly and to tell others that this resource exists. People may post this content online or redistribute in any way for non-commercial purposes.

Read more about Early Journal Content at <http://about.jstor.org/participate-jstor/individuals/early-journal-content>.

JSTOR is a digital library of academic journals, books, and primary source objects. JSTOR helps people discover, use, and build upon a wide range of content through a powerful research and teaching platform, and preserves this content for future generations. JSTOR is part of ITHAKA, a not-for-profit organization that also includes Ithaka S+R and Portico. For more information about JSTOR, please contact support@jstor.org.

AMERICAN JOURNAL OF BOTANY

VOL. IX

DECEMBER, 1922

NO. 10

THE EFFECT OF BORDEAUX MIXTURE UPON THE CHLOROPHYLL CONTENT OF THE PRIMOR- DIAL LEAVES OF THE COMMON BEAN, *PHASEOLUS VULGARIS* L.

W. A. RUTH

(Received for publication March 4, 1922)

INTRODUCTION

To the quantitative relations and specific function of chlorophyll is to be attributed the faculty of the plant to produce much or little organic material under the prevailing conditions. Our knowledge does not enable us to make a final statement concerning the difference in chlorophyll in different individuals or species, but the results of recent and earlier investigations would lead us to the belief that the difference in performance, that is, in the ability to produce organic matter, rests largely, if not entirely, with the protoplast and depends upon the quantity, rather than the quality, of chlorophyll.

Until Willstätter and Stoll (1913) published a method of analysis, the absolute quantity of chlorophyll per unit area of leaf tissue could not be determined. Thus, Rosé (1913, p. 29) compared leaf extracts with a standard extract of unknown value.

La chlorophylle n'ayant pas été isolée, on ne peut, comme le fait observer Lubimenko, procéder à des déterminations de quantités absolues. Il faut se contenter de la détermination de quantités relatives à un étalon.

A plan for a systematic quantitative study of the chlorophyll content of foliage leaves is part of a general project of the laboratory of plant physiology of the University of Illinois. This paper is limited, for the most part, to a study of the effects of Bordeaux mixture; other relations were studied more or less incidentally, these consisting of the relation of the amount of chlorophyll to the age of the leaves and to the rapidity of their growth.

The studies are to be prosecuted along two general lines: First, a study of the quantitative relation of the chlorophyll content of plants treated in various ways but growing under the general external conditions which

[The *Journal* for November (9 : 471-533) was issued November 27, 1922].

practical greenhouse men now advise for vegetable growing. Such conditions, in the main, approach very closely the ecological optimum (Schimper, 1903, p. 44). Second, a study of the chlorophyll content of leaves as affected by the shifting of one or more of the ecological factors, as light, temperature, or moisture, independently or together, in a plus or minus direction, etc. The second plan involves long and difficult experimentation if the results are to be of permanent value. It becomes necessary to provide for the strict regulation of each environmental factor or factors working together or in opposition. The variation in effect of a single factor under widely varied conditions has been clearly brought out by Livingston (1917a, p. 6). The work reported in this paper is based on the former method. I wish to thank Professor Charles F. Hottes, of the Department of Botany of the University of Illinois, for his advice and help, which he gave me at every stage of this work.

It is a well-established fact that a large number of plants sprayed with Bordeaux mixture show a distinctly greener color. This would lead one to expect an increase in the chlorophyll content of sprayed leaves. The deeper green of sprayed as contrasted with unsprayed leaves might be brought about by an increase in the quantity of chlorophyll in the plastids, or by an increase in the number of plastids, or in both ways. It might, on the other hand, be due to a smaller size of individual cells, and consequently to the crowding of the normal number of plastids into a smaller area. To my knowledge no quantitative experiments have been made to measure the increase in chlorophyll content of sprayed leaves, if such an increase takes place.

That the various effects of Bordeaux mixture are due to the copper ion has been the conclusion of most, but not of all, investigators. Frank (1888, p. 535) found that distilled water had a toxic effect upon the roots of lupines. Loew (1891) attributed the poisonous properties of distilled water to traces of copper in solution. Nägeli (1893) found that water in a glass vessel in which copper coins had been placed had a toxic effect upon *Spirogyra*. Because of the extreme dilution he thought that the action of the copper was not chemical, but due to some other force, which he described as "oligodynamic." Rumm (1893), because he was unable to find traces of copper in leaves affected physiologically by Bordeaux mixture, and Frank and Krüger (1894), because they thought that too little copper was in solution in the fungicide to act on fungous spores, applied Nägeli's hypothesis of oligodynamic action. Millardet and Gayon (1885) found that the normal germination of spores of *Peronospora viticola* would not take place at a higher concentration of copper sulphate than three parts in ten million. They found later (1887) that the cuticle of leaves had the power of removing copper from a solution of copper sulphate; they believed that copper was taken out of solution in this way from Bordeaux mixture and actually penetrated the cells. Crandall (1909, p. 230) objects to the methods of

Millardet and Gayon because of the large amounts of soluble copper they used in their experiments and the insolubility of the Bordeaux precipitate. Pickering (1910, pp. 113-115) concludes from his experimental work that penetration takes place, and that the carbon dioxide of the air is the dissolving agent (1910, pp. 27-36). Bain's very careful investigations (1902) have sustained the hypothesis of penetration. Bain (1902, p. 88) states his conclusions as follows:

From all the evidence presented on the preceding pages, there can remain little doubt that copper is absorbed by the leaves of plants sprayed with Bordeaux mixture. . . . Of course, the most conclusive and only final evidence of the entrance of the copper into the tissues of the leaf is to demonstrate its presence there by an appropriate test. The writer has made no experiments in this direction.

The belief of Millardet and Gayon in the actual penetration of copper receives further support from Schander (1904) and Ewert (1905). Schander believed, however, that the action of rain and dew is seldom of direct importance in dissolving copper salts from Bordeaux mixture because of the insolubility of the precipitate, and that in many plants, *e.g.*, in the bean and apple, the entrance of copper occurs through the hairs as the result of their excretion of an alkaline substance, or through glands in the case of other plants, like *Fuchsia* and *Oenothera*, following the excretion of acidic substances.

That the physiological effects of Bordeaux mixture are due to the penetration of copper, not only in solution but in solution in the ionic state, seems very probable if we consider the indirect evidence supplied by the work of Kahlenberg and True (1896) and Heald (1896), in connection with that of Bain (1902), Clark (1902), and Pickering (1908, 1910). Kahlenberg and True, and Heald, working with seedlings in very dilute solutions of copper salts, proved that the toxicity of the dissolved copper salt was directly proportional to the concentration of copper ions in the solution. Bain (1902, pp. 42-52) showed that sprayed peach foliage is not injured unless liquid water is present on the leaf. One would expect these results in view of the probable effect of the above-named substances in increasing or decreasing the concentration of copper ions in the Bordeaux suspension. Bain also showed (1902, pp. 36-44) that certain salts, for example, calcium chloride, calcium sulphate, and calcium nitrate, produce an increased Bordeaux injury, while lime and various carbonates have a tendency to prevent it. Clark (1902) and Pickering (1910, pp. 143-145) have shown that potassium sulphate decreases the toxicity of copper sulphate, and Pickering (1908, p. 106) has shown that sodium chloride increases it. Potassium sulphate and sodium chloride would modify the ionization of solutions of copper sulphate in a manner corresponding to the decreased and increased injury.

Besides the change in the concentration of copper ions, antagonisms, such as that of copper and calcium which has been demonstrated by True

and Gies (1903) and Hawkins (1913), may have been involved in the effect of additions of other salts. As a result of the work of Boussingault (1878) and Bain (1902), there can be little doubt that the relatively insoluble calcium salts found in Bordeaux mixture can penetrate the leaf.

Duggar and Bonns (1918), in a study of the effects of spraying with Bordeaux mixture on transpiration, offer as an explanation of its effect on this particular process an entirely different hypothesis: The Bordeaux mixture, which Duggar and Cooley (1914) regard as a film, acts as a bibulous material, taking water directly from the interior of the plant. This explanation is based on the facts, as observed by them, that xerophytic plants show no increased transpiration when sprayed, and that the increase occurs, in the case of mesophytes, at night only, at which time the stomata are probably suffused with water.

The manner of action of Bordeaux mixture has remained unsolved. The importance of the fact that the presence of the precipitate in contact with the solution assures a constant supply of dissolved copper salts has been brought out by Pickering (1910, pp. 6-9). The low concentration can not play the part in Bordeaux mixture that it plays in the case of a solution not in contact with the solid phase, where the concentration, as Clark (1902) has shown, is important. This fact should overcome some of the objections to the penetration theory.

MATERIALS AND METHODS

Plants of *Phaseolus vulgaris* (variety Dreer's Extra Early Refugee) were used in all the experiments. The seed was obtained in one lot from Vaughan's Seed Store, Chicago, Illinois. The plants were grown in the greenhouse, in a uniformly mixed and sifted Urbana brown silt-loam, in flats three inches deep, ten and one half inches wide, and twenty-two and one half inches long (inside measurements).

In planting, the slightly moist soil was sifted into the flats and all soil within one inch of the top was removed. The beans were laid on this soil two inches apart in the row and with four inches between the rows. One inch of soil was then added, smoothed off, and evenly compacted. The flats were watered and covered until the seedlings appeared above the soil, and the latter were then transferred to rotating tables similar to those described by Livingston (1917b, p. 149).

The rotating tables were located in the center of a south room in the greenhouse. The tripods were set on widely spaced two-inch boards, which were supported on a solid frame made of iron pipes. The nature of the foundation and the height of the platforms above the ground (four feet) secured a free circulation of air between the flats, and, together with the distance from the heating pipes (six feet), guarded against uneven heating and uneven relative humidity. Power was transmitted from the

motor to the tables by a belt. The motor was located on a separate stand to reduce vibration. The period of rotation of the tables was six and one half minutes.

The steam heat of the greenhouse was under thermostatic control, maintained at twenty degrees C. The humidity of the air was controlled by flooding the broad trenches under the slatted walks and benches each morning. At this time the flats were watered. On cloudy days the tables were artificially illuminated by stage flood lights, each of which was provided with a 1000-watt nitrogen-filled tungsten lamp. The distance from the lamps to the tables was five feet. The effectiveness of this additional illumination as an aid to the normal growth of the plants (Lubimenko, 1905) is indicated by the prevention of unusual elongation of the stem on such days. The results obtained by this artificial lighting may be illustrated by the following: December 13, 15, and 16 were very cloudy. On December 13, the cotyledons of the young plants were just appearing above the soil of two flats. One of these was artificially illuminated by means of a flood light; the other, similarly located, received daylight only. The length of the stem to the primordial leaves of the former averaged 9 cm. on December 16 (individual measurements, 10, 9, 13, 10, 9, 9, 6, 6, 6, 12, 9, 6, 11, 9, and 9 cm.), while the stems of the latter averaged 15 cm. (individual measurements, 16, 16, 13, 16, 15, 14, and 16). (Cf. Johnston, 1917.)

As soon as the primordial leaves had unfolded the plants were thinned out. From twelve to twenty-four plants of uniform appearance and growth, and as evenly spaced as possible, were left in each flat. In the experiments on the effect of spraying with Bordeaux mixture, the plants in one half of each flat were sprayed; in the experiments on the relation of the chlorophyll content to the rapidity of growth of the primordial leaves, disbudding, by which the increased rate of growth of the primordial leaves was secured, was carried out on alternate plants. In the experiments on the effect of age, care was taken to maintain an equal spacing as plants were removed during the progress of the experiment.

The results recorded are based upon data secured through the use of the primordial leaves only. No selection of plants or leaves was made at harvesting. The leaves were measured and weighed in the fresh condition and immediately dried. The areas were determined by drawing the outline of the leaves on weighed letter-size sheets of Hammermill Bond paper, and weighing the enclosed portion of the sheet. The paper was found to be remarkably uniform in weight; nevertheless, each sheet was weighed separately. The leaves were dried in a rapid current of air at a temperature of from forty-five to fifty degrees C. About an hour was required for average-sized leaves. During drying, the leaves were protected from sunlight. As soon as the leaves were crisp, each lot was stored in a small tin box until its chlorophyll content was determined.

The manner of determining chlorophyll consisted in the comparison in a Duboscq colorimeter of 85-percent acetone extracts made from 0.25-gram samples of these leaves after thorough powdering in a mortar, with a standard extract made in the same manner from a bean-leaf powder which had been standardized by quantitatively determining its chlorophyll content.

The quantitative determination of chlorophyll by the use of these raw acetone solutions, which contained all four pigments, chlorophyll *a*, chlorophyll *b*, carotin, and xanthophyll, was possible because the shade of color of all the solutions closely matched that of the solution used as a standard.

Die Rohchlorophylllösung enthält die vier Pigmente von verschiedener Farbe und Farbintensität in Mengenverhältnissen, die von Lösungsmittel beeinflusst werden und die von der Pflanzenart und sogar von der Ernte einer und derselben Pflanze abhängig sein können. *Annähernd gleich ist das Mengenverhältnis der Komponenten bei den Extrakten einer Pflanze; man kann daher durch den Vergleich derselben die relative Bestimmung ihres Chlorophyllgehaltes ausführen.* Für die Untersuchung von Extrakten ungleicher Farbnuance, also z.B. aus verschiedenen Pflanzen, ist es erforderlich, die Farbstoffe durch Verseifung mit Alkali in die indifferenten gelben und in die Alkalisalze der grünen Pigmente zu trennen. Die erhaltenen Chlorophyllinlösungen ermöglichen die relative Bestimmung des Farbwertes und ferner erlaubt ihr Vergleich mit einer alkoholischen Lösung von bekanntem Chlorophyllgehalt die absolute Bestimmung des Farbstoffgehalts. (Willstätter and Stoll, 1913, pp. 78, 79.)

The primordial leaves used in making the standard powder were obtained from beans grown in the greenhouse from the same lot of seeds used in growing the plants for the experiments.

In carrying out the quantitative determination of chlorophyll in the powder to be used as a standard, an indirect process is necessary, because mechanical difficulties interfere with quantitatively separating chlorophyll as a solid. The process is, briefly, as follows: Chlorophyll (the mixture of chlorophyll *a* and chlorophyll *b*) is obtained from a rather large but not exactly weighed quantity of the powder and dried. A quantitatively weighed amount of this chlorophyll is dissolved in absolute alcohol and made up to a definite volume. A quantitatively weighed amount of the leaf powder from which the chlorophyll has been obtained is extracted, the carotin and xanthophyll are removed from the extract, and the resulting solution, made up to a definite volume (in water and alcohol), is compared colorimetrically with the solution made directly from the weighed chlorophyll. To facilitate removing carotin and xanthophyll from the extract from the powder, the chlorophyll *a* and chlorophyll *b* are saponified, giving the corresponding chlorophyllins (chlorophyllin *a* and chlorophyllin *b*) in unchanged proportion. The saponification and resulting comparison of chlorophylls and chlorophyllins does not introduce an error, according to Willstätter and Stoll (1913, p. 82).

To isolate the chlorophyll the following steps are necessary: (1) complete extraction of the leaf powder with eighty-five percent acetone; (2) transference of the pigments to petrol ether; (3) removal of the greater part of

the acetone by washing with water; (4) separation of xanthophyll by washing with eighty percent methyl alcohol; (5) removal of acetone and methyl alcohol by washing with water, which results in the precipitation of the chlorophyll; (6) removal of the water by means of anhydrous sodium sulphate; (7) removal of the fine precipitate of chlorophyll and its separation from carotin by filtering through talc; (8) solution of the precipitated chlorophyll in ether and reprecipitation by adding petrol ether; (9) filtration through talc; (10) solution of the precipitate in ether; (11) evaporation to dryness. (The processes are described and discussed in detail by Willstätter and Stoll, 1913, pp. 132-135.)

The chlorophyllin solution was made by (1) transferring the pigments in a raw eighty-five-percent acetone extract to ether; (2) saponification of the two chlorophylls with methyl-alcoholic potash; (3) the separation of these products from carotin and xanthophyll by extracting them with water. (Willstätter and Stoll have given complete directions for this separation, 1913, pp. 81-82.)

The only modification of the method presented by Willstätter and Stoll was the use of comparatively larger quantities of solvent than the small quantities of leaf powder called for. The entire amount of leaf powder to be standardized was mixed and quartered, and from these portions were taken 0.25-gram samples for the preparation of the raw solutions in 85-percent acetone, to be used as standards, 10 grams for the isolation of the solid chlorophyll mixture, and 2.5-gram samples for the preparation of the chlorophyllins.

The samples used for chlorophyllin preparation were extracted with 85-percent acetone and made up to 100 cc. Ten cc. of this were used in the succeeding steps. A solution made by diluting the result of the final separation to 100 cc. with alcohol was of the proper strength for comparison with the standard chlorophyll solution of Willstätter and Stoll. (0.0513 gram chlorophyll is dissolved and made up to 100 cc. in absolute alcohol. For use in the colorimeter 10 cc. are diluted to 200 cc. 1913, p. 81.)

In making a raw extract for colorimetric comparison a 0.25-gram sample was extracted with successive small quantities of 85-percent acetone, which were filtered off, combined, and made up to 50 cc. in a graduated flask. Portions of approximately 2 cc. were used in the colorimeter. The depth of the standard raw acetone extract in the colorimeter was maintained at 20 mm.

VARIATION IN CHLOROPHYLL CONTENT WITH DEVELOPMENT OF THE PRIMORDIAL LEAVES

Before determining the effect of spraying upon the chlorophyll content, it was thought desirable to determine the degree of variation in chlorophyll content which took place as the plant grew. Willstätter and Stoll (1915)

have shown that the chlorophyll content of leaves of certain species increases until the leaf is mature, after which it decreases. Possibly the decrease in chlorophyll may be attributed to the breaking down of the proteins of the chloroplasts, due to a shortage of carbohydrates in the plant and their use as a source of this material (Meyer, 1918), or to a shortage of nitrogen (Schertz, 1919). Palladin (1891) has shown definitely that a supply of soluble carbohydrates is necessary for the formation of chlorophyll, and it was thought that, aside from the age factor, the presence and dropping of the cotyledons might be involved. Their loss might mark a point of change in the chlorophyll content of the primordial leaves, caused possibly by the decreased supply of nitrogen or of carbohydrates or of both. Such changes might, it was thought, be great enough to overshadow or counterbalance any effect of spraying, although the possibility remained that they would emphasize it. Their importance in the growth of *Phaseolus vulgaris* is shown by the work of Lüpke (1888), and in the growth of the Canada field pea by Duggar (1919), who assumes that they may be a source of organic nitrogenous material or of a vitamine.

The chlorophyll content of the primordial leaves was determined at the following four stages of development: "Age A," immediately after the primordial leaves had unfolded; "Age B," when the cotyledons were being shed, three days after "Age A"; "Age C," four days after "Age B," when the primordial leaves had attained a considerable size, but while their area still constituted the greater part of the photosynthetic surface of the plant; and "Age D," when the primordial leaves constituted only a small part of the total leaf surface but before they showed yellowing. Seventeen days elapsed between "Age C" and "Age D."

The data for "Age A" were obtained by measuring and weighing the primordial leaves of thirty-two plants; the primordial leaves from twenty plants were used to obtain the data for "Age B"; those from sixteen plants for "Age C"; and those from twenty plants for "Age D." The results are shown in table 1.

TABLE 1. *Chlorophyll Content of Primordial Leaves at Various Ages*

| Age | Area Both Primordial Leaves | | Fresh Wt. Leaf per sq. cm. (g.) | Wt. Chlorophyll (mg.) | |
|----------|-----------------------------|------------|---------------------------------------|-----------------------|------------------|
| | Sq. cm. | % Increase | | Per sq. cm. | Per g. Fresh Wt. |
| "A"..... | 23.4 | | .0241 | .0305 | 1.26 |
| "B"..... | 64.6 | 176. | .0186 | .0412 | 2.22 |
| "C"..... | 107.8 | 67. | .0201 | .0395 | 1.96 |
| "D"..... | 146.0 | 35. | .0219 | .0388 | 1.77 |

During the period of growth before the cotyledons were shed, which was also the period of most rapid expansion, there was a marked increase in the amount of chlorophyll to the square centimeter. The increase to

the gram of fresh weight was still more marked. From this period on there was a decrease in the amount of chlorophyll to the square centimeter and gram of fresh weight, which was associated with an increase in the fresh weight of the leaf per square centimeter and a lessened rate of increase in area. This decrease was not so great that neutralization of any effect of early spraying was anticipated before "Age D" was reached. The necessity was recognized of comparing plants harvested only at the same stage of development.

The correlation between rapid growth and high chlorophyll content was determined also in the following way: To accelerate and increase the growth of the primordial leaves of certain plants, the growing buds above them were kept cut off. The primordial leaves of such plants became much larger, thicker, and greener than the primordial leaves of the check plants among them. Measurements of the area and weight were made when the plants were picked, which was four days after the cotyledons were shed

TABLE 2. *Effect of Removal of Growing Buds upon Growth and Chlorophyll Content of Primordial Leaves*

| Flat | Average Area Both Leaves | | | Average Weight Both Leaves per sq. cm. | | | Average Chlorophyll per sq. cm. | | | Average Chlorophyll per g. Fresh Wt. | | |
|------|--------------------------|---------|--------------|--|--------|--------------|---------------------------------|-----------|--------------|--------------------------------------|-----------|--------------|
| | Check | | Buds Removed | Check | | Buds Removed | Check | | Buds Removed | Check | | Buds Removed |
| | Sq. cm. | Sq. cm. | % of check | G. | G. | % of check | Wt. (mg.) | Wt. (mg.) | % of check | Wt. (mg.) | Wt. (mg.) | % of check |
| 1 | 76.9 | 119.9 | 154 | 0.0181 | 0.0225 | 124 | 0.0315 | 0.0454 | 144 | 1.74 | 2.02 | 116 |
| 2 | 83.2 | 112.1 | 135 | 0.0186 | 0.0241 | 129 | 0.0353 | 0.0524 | 148 | 1.89 | 2.17 | 115 |
| 3 | 93.2 | 127.7 | 137 | 0.0199 | 0.0251 | 126 | 0.0381 | 0.0527 | 138 | 1.91 | 2.10 | 110 |
| Ave. | | | 142 | | | 126 | | | 143 | | | 114 |

("Age C"). The leaves from the plants similarly treated in each flat were dried, and their chlorophyll content was determined together. The figures presented in table 2 are half-flat averages. Three flats were used, in which forty-two plants were grown. A greater chlorophyll content per square centimeter was plainly produced, which was to be accounted for only partly on the basis of a greater weight of a unit area of leaf; the average increase in the chlorophyll per square centimeter was 143 percent, while the weight of the leaf per square centimeter was increased only 126 percent. An actual increase in the amount of chlorophyll in a unit weight of fresh leaf (114 percent) was thus an important factor. The average increase in chlorophyll per square centimeter (143 percent) corresponded with the average increase of 142 percent in the rate of expansion of the whole leaf in area.

THE EFFECT OF BORDEAUX MIXTURE

The experiments immediately following were to determine, by measuring the length of sprayed and unsprayed leaves, (1) how soon after spraying young primordial leaves, just unfolding, the effect upon growth was to be observed; (2) whether the growth rate was increased or decreased; (3) when the effect was most manifest; and (4) whether any such effect was outgrown. A quickly manifested effect was anticipated in view of the rapid growth of the leaves and the quickly manifested effect of Bordeaux mixture upon transpiration (Martin, 1916).

Five flats were planted to beans, one flat at a time, at intervals of from one to five days. The total period of planting covered eighteen days, and the plants were exposed, on that account, to slight variations in weather conditions. As soon as the primordial leaves had unfolded, the plants in one half of each flat were sprayed with Bordeaux mixture. The length of the leaves was determined at that time and at intervals of two, four, eight, twelve, and fourteen days after spraying. Measurements made on the fourteenth day gave the same average lengths as those made on the twelfth. No measurements made after the twelfth day are presented.

Although the average length of the sprayed leaves in three of the five flats somewhat exceeded the length of the leaves not to be sprayed when the experiment was started, the sprayed leaves in all the flats were the shorter at the conclusion of the experiment. The effect in decreased growth was plainly evident on the second day, and probably could have been observed earlier. On the fourteenth day the relative differences to be observed on the second day had not been overcome. The results are summarized in table 3.

TABLE 3. *Effect of One Application of Bordeaux Mixture as Shown by a Lessened Growth in Length*

| Days after Spraying ¹ | Treatment | Average Length Primordial Leaves (cm.), Flat Number | | | | | Relation of Length of Sprayed to Unsprayed Leaves in Percentages |
|-------------------------------------|----------------|--|-----|-----|-----|-----|--|
| | | 1 | 2 | 3 | 4 | 5 | |
| 0..... | Sprayed..... | 6.1 | 4.3 | 4.6 | 3.9 | 3.3 | 103 |
| | Unsprayed..... | 6.1 | 4.1 | 4.4 | 3.6 | 3.4 | |
| 2..... | Sprayed..... | 6.8 | 5.4 | 5.9 | 5.5 | 4.8 | 97 |
| | Unsprayed..... | 7.1 | 5.6 | 5.9 | 5.8 | 4.9 | |
| 4..... | Sprayed..... | 7.2 | 6.4 | 6.8 | 6.6 | 5.9 | 96 |
| | Unsprayed..... | 7.7 | 6.5 | 6.9 | 6.9 | 6.3 | |
| 8..... | Sprayed..... | 7.6 | 7.0 | 7.6 | 7.7 | 6.3 | 96 |
| | Unsprayed..... | 8.2 | 7.2 | 7.8 | 7.8 | 6.5 | |
| 14..... | Sprayed..... | 7.7 | 7.3 | 7.7 | 7.9 | 7.2 | 95 |
| | Unsprayed..... | 8.3 | 7.4 | 7.9 | 8.3 | 7.8 | |

¹ The state of development of the plant when the leaves were sprayed is represented by "Age A" in the preceding section; the fourth day corresponds with "Age B," and the eighth day with "Age C."

It was apparent that if Bordeaux mixture resulted in a change in chlorophyll content correlated with a decreased growth rate, one might expect such a change to be revealed soon after the spray was applied. It was concluded from the results of the above-described experiment that any differences in chlorophyll content brought about by the action of Bordeaux mixture applied early in the growth of the leaf would probably be preserved rather than materially lessened.

The effect of Bordeaux mixture upon the development of chlorophyll may be indicated by reporting an experiment in which a set of six flats, planted on the same day, was used. The primordial leaves of one half of the plants in three of these flats were sprayed as soon as they had unfolded (flats 1, 2, and 3 in tables 4-7). The same treatment was given to the primordial leaves of one half of the plants in the other three flats when the cotyledons were dropping (flats 4, 5, and 6 in the same tables), four days later. The plants in all six flats were harvested on the same day, three days after the later application, and seven days after the earlier one.²

At the time of spraying the average length of the sprayed leaves in all the flats was 5.7 cm., while those not to be sprayed averaged 5.9 cm. Flat averages of the areas and chlorophyll contents per square centimeter when the leaves were picked are presented in table 4.

TABLE 4. *Average Areas and Chlorophyll Content per Square Centimeter of Sprayed and Unsprayed Leaves*

| Flat | Area One Leaf (sq. cm.) | | Chlorophyll (mg. per sq. cm.) | |
|------|-------------------------|-------------|-------------------------------|-------------|
| | Sprayed | Not Sprayed | Sprayed | Not Sprayed |
| 1 | 48.7 | 63.9 | .00436 | .00330 |
| 2 | 54.7 | 62.4 | .00375 | .00401 |
| 3 | 57.2 | 53.7 | .00382 | .00371 |
| 4 | 42.6 | 45.9 | .00442 | .00399 |
| 5 | 61.2 | 63.9 | .00340 | .00304 |
| 6 | 49.6 | 57.6 | .00363 | .00356 |
| Ave. | 53.3 | 57.9 | .00390 | .00361 |

The chlorophyll content of unit areas of the sprayed leaves from all the flats with the exception of those from flat 2 was greater than that of the corresponding unsprayed leaves. The areas of the sprayed leaves were less than the areas of those not sprayed, with the exception of those from the plants grown in flat 3. The general results were anticipated in view of the darker color of the sprayed foliage and the stunting effect of the spray which had been manifested in the previous experiment in a lessened increase in length.

The darker color was correlated with this smaller size. In spite of the lessened rate of expansion in area brought about by spraying, the chloro-

² This stage of development is referred to as "Age C" in the preceding section, where the state of unfolding is designated as "Age A" and that of cotyledon shedding as "Age B."

phyll content of the average sprayed and of the average unsprayed leaf was practically the same, averaging 2.02 milligrams to the sprayed leaf and 2.07 milligrams to the leaf which had not been sprayed. Averages for the six flats are presented in table 5.

TABLE 5. *Average Chlorophyll Content (in Milligrams) of the Primary Leaves of Sprayed and Unsprayed Plants*

| Flat | 1 | 2 | 3 | 4 | 5 | 6 | Average |
|----------------|------|------|------|------|------|------|---------|
| Sprayed..... | 2.12 | 2.05 | 2.18 | 1.88 | 2.08 | 1.80 | 2.02 |
| Unsprayed..... | 2.11 | 2.50 | 1.99 | 1.83 | 1.94 | 2.05 | 2.07 |

This was not due to an increase in the weight of the sprayed leaf per unit area, as table 6 shows.

TABLE 6. *Weight of Sprayed and Unsprayed Leaves (in Grams) per Square Centimeter*

| Flat..... | 1 | 2 | 3 | 4 | 5 | 6 | Average |
|----------------|-------|-------|-------|-------|-------|-------|---------|
| Sprayed..... | .0204 | .0187 | .0187 | .0164 | .0185 | .0189 | .0186 |
| Unsprayed..... | .0189 | .0204 | .0195 | .0157 | .0189 | .0193 | .0189 |

The primordial leaves of the sprayed plants in all the flats contained the greater amount of chlorophyll per gram of fresh leaf. The data are presented in table 7.

TABLE 7. *Chlorophyll Content (in Milligrams) per Gram, Fresh Weight*

| Flat..... | 1 | 2 | 3 | 4 | 5 | 6 | Average |
|----------------|------|------|------|------|------|------|---------|
| Sprayed..... | 2.14 | 2.00 | 2.04 | 2.30 | 1.82 | 2.00 | 2.05 |
| Unsprayed..... | 1.75 | 1.97 | 1.96 | 2.17 | 1.61 | 1.86 | 1.89 |

DISCUSSION

Probable Relations between Photosynthesis and the Increased Chlorophyll Content. It has been shown that the chlorophyll content of unit areas and of unit fresh weights has been increased by spraying rapidly growing leaves with Bordeaux mixture. This increase is accompanied by a smaller leaf area. No experiments were carried out to determine the effect upon photosynthesis; this is one of a series of topics outlined for further experiment. Amos (1907), experimenting with leaves of the hop, grape, and Jerusalem artichoke, found that Bordeaux mixture temporarily checked photosynthesis. After the effect of the temporary check had disappeared, photosynthesis became normal but did not exceed that of unsprayed leaves. No effect of the spray on the color of the leaves was reported, which prevents the direct application of his conclusions to this experiment.

If whole leaves had been taken as units, and if an examination had been made at "Age C," it is possible that the photosynthetic activity of the sprayed leaves would have been found to be about the same as that of unsprayed leaves, because the chlorophyll content of the sprayed and unsprayed leaves was approximately equal. On the other hand, the effect of the Bordeaux mixture upon other conditions involved in photosynthesis might prove such an assumption to be groundless. In fact, it is more probable that photosynthesis was decreased; at any rate, in the experiment reported above on the effect of disbudding, a direct relation was shown between a high chlorophyll content of unit areas and a rapid increase in the area of the whole leaf, while the same positive relation between the chlorophyll content and the growth rate held at the various ages. Moreover, the decreased respiration produced by Bordeaux mixture under conditions similar to those of this experiment (Ewert, 1905) might indicate a lowered photosynthetic activity, since a positive relation between photosynthesis and respiration, except under extreme conditions, has been proven by Spoehr and Long (1919). Plester (1912) concludes that structure, which is evidently changed by Bordeaux mixture, is a factor in the rate of photosynthesis of varieties of the same species differing in their chlorophyll content, and Rosé (1913, p. 105) arrives at the same conclusion as the result of his work on the photosynthetic rate manifested by leaves developed under different conditions of illumination. Another factor which might be affected is the activity of an enzyme to which Willstätter and Stoll (1915) attribute the high photosynthetic activity of very young, etiolated, and chlorotic leaves in which the photosynthetic rate is much above the rate indicated by their chlorophyll content. Certain investigators, including Frank and Krüger (1894) and Bain (1902), have found greater amounts of starch in the leaves of plants treated with relatively insoluble copper compounds, including Bordeaux mixture. These investigators have concluded that photosynthesis has been increased. Ewert (1905), however, finds that the yield is always lowered by Bordeaux mixture, whether it is measured in starch, in protein, or in dry weight. He finds that respiration is decreased and the rate of translocation of carbohydrates is lessened, the latter effect accounting for the apparent increase in assimilation observed by earlier investigators. He attributes the decreased growth which he obtained to a poisoning exerted on various activities of the plant, including diastatic activity.

Aside from the harmful effect upon photosynthesis, which Bordeaux mixture may have in ways like those above suggested, it would seem that the increase in transpiration which it evidently brings about would be harmful to growth, unless the water content of the growing cells is maintained, possibly by an increased activity in water absorption by the root.

Environmental Conditions. The conditions for the growth of the plants used in this experiment were extremely favorable to rapid and normal

development. Extremes were avoided, and it is probably due to the care exercised that injuries such as those reported by Frank and Krüger (1894) were not observed. These authors reported a severe Bordeaux injury to the foliage of potatoes as the result of adverse conditions, including very high temperatures. Bain (1902) also states that high temperature is a factor in the physiological response of peach foliage to copper salts, being manifested in both increased assimilation and increased injury. Crandall (1909) states that low vitality is a factor in the production of injury to the foliage of the apple following the application of Bordeaux mixture. The freedom of the plants from injury may have been due in part to the fact that the foliage was not wetted, which, it is frequently stated, is a necessary condition, although Schander (1904) has shown that bean foliage can be injured by Bordeaux mixture without the presence of liquid water. Under conditions brought about by too low temperatures or too little illumination it is possible that the leaves of the bean would show no appreciable response.

SUMMARY

1. The primordial leaves of the bean sprayed with Bordeaux mixture do not grow to the size attained by unsprayed leaves.
2. A retardation in growth is manifested very soon after the spray is applied. The relative difference is maintained.
3. The chlorophyll content per unit area of the primordial leaves of the bean is slightly increased by spraying with Bordeaux mixture.
4. The chlorophyll content per unit area of the primordial leaves of the bean decreases as the leaves develop after the shedding of the cotyledons.
5. The chlorophyll content per unit area of the young primordial leaves of the bean is lower than that of the primordial leaves of the bean just after the shedding of the cotyledons.
6. The primordial leaves of the bean increase in area and in weight per unit area following the suppression of the growing buds.
7. This increase in area and weight per unit area is accompanied by a disproportionately greater increase in the chlorophyll content per unit area.

BIBLIOGRAPHY

- Amos, A. 1907. The effect of fungicides upon the assimilation of carbon dioxide by green leaves. *Jour. Agr. Sci.* 2: 257-266.
- Bain, S. M. 1902. The action of copper on leaves. *Tenn. Exp. Sta. Bull.* 15²: 1-108.
- Bell, J. M., and Taber, W. C. 1907. The action of lime in excess on copper sulphate solutions. *Jour. Phys. Chem.* 11: 632-638.
- Boussingault, J. 1878. Étude sur les fonctions physiques des feuilles. *Annales Chim. Phys.* V, 13: 289-394.
- Clark, J. F. 1902. On the toxic properties of some copper compounds with special reference to Bordeaux mixture. *Bot. Gaz.* 33: 26-48.
- Coupin, H. 1898. Sur la toxicité du chlorure de sodium et de l'eau de mer e l'égard des végétaux. *Rev. Gén. Bot.* 10: 177-190.
- Crandall, C. S. 1909. *Ill. Exp. Sta. Bull.* 135.

- Duggar, B. M. 1919. Nutritive value of food reserve in cotyledons. Yearbook Carnegie Inst. Wash. 18: 81, 82.
- , and Bonns, W. W. 1918. The effect of Bordeaux mixture on the rate of transpiration. Ann. Mo. Bot. Gard. 5: 153-176.
- , and Cooley, J. S. 1914. The effect of surface films and dusts on the rate of transpiration. Ann. Mo. Bot. Gard. 1: 1-22.
- Ewert, R. 1905. Der wechselseitige Einfluss des Lichtes und der Kupferkalkbrühen auf den Stoffwechsel der Pflanze. Landw. Jahrb. 34: 233-310.
- Frank, B. 1888. Untersuchungen über die Ernährung der Pflanze mit Stickstoff. Landw. Jahrb. 17: 420-554.
- , and Krüger, F. 1894. Ueber den Reiz, welchen die Behandlung mit Kupfer auf die Kartoffelpflanze hervorbringt. Ber. Deutsch. Bot. Ges. 12: 8-11.
- Galloway, B. T. 1890. Report of the chief of the section of vegetable pathology, U. S. Dept. Agr., for the year 1889.
- Hawkins, L. A. 1913. The influence of calcium, magnesium and potassium nitrates upon the toxicity of certain heavy metals toward fungus spores. Physiol. Res. 1: 57-92.
- Heald, F. D. 1896. The toxic effect of dilute solutions of acids and salts upon plants. Bot. Gaz. 22: 125-153.
- Johnston, E. S. 1917. Seasonal variations in the growth-rates of buckwheat plants under greenhouse conditions. Johns Hopkins Univ. Circ. n. ser. 3: 211-217.
- Kahlenberg, L., and True, R. H. 1896. On the toxic action of dissolved salts and their electrolytic dissociation. Bot. Gaz. 22: 81-124.
- Livingston, B. E. 1917a. A quarter-century of growth in plant physiology. Plant World 20: 1-15.
- , 1917b. The Department of Plant Physiology. Johns Hopkins Univ. Circ. n. ser. 3: 133-159.
- Loew, O. 1891. Bemerkung über die Giftwirkung des destillierten Wassers. Landw. Jahrb. 20: 235.
- Lubimenko, W. 1905. Sur la sensibilité de l'appareil chlorophyllien des plantes ombrophiles et ombrophobes. Rev. Gén. Bot. 17: 381-415.
- Lüpke, R. 1888. Ueber die Bedeutung des Kaliums in der Pflanze. Landw. Jahrb. 17: 887-913.
- MacDougal, D. T. 1919. Growth and hydration. Yearbook Carnegie Inst. Wash. 18: 57-60.
- Martin, W. H. 1916. The influence of Bordeaux mixture on the rates of transpiration from abscised leaves and from potted plants. Jour. Agr. Res. 7: 529-548.
- Meyer, A. 1918. Eiweissstoffwechsel und Vergilben der Laubblätter von *Tropaeolum majus*. Festschrift zum Ernst Stahl, pp. 85-127. Jena, 1918. Abstract by W. Crocker. Bot. Gaz. 67: 446, 447. 1919.
- Millardet, A., and Gayon, U. 1885. Recherche du cuivre sur les vignes traitées par le mélange de chaux et de sulfate de cuivre et dans la récolte. Jour. Agr. Prat. 49²: 732-734.
- , 1887. Jour. Agr. Prat. 51¹: 123-129, 156-161.
- von Nägeli, C. 1893. Ueber oligodynamische Erscheinungen in lebenden Zellen. Denkschr. Schweiz. Naturf. Ges. 33: 1.
- Palladin, W. 1891. Ergrünen und Wachstum der etiolierten Blätter. Ber. Deutsch. Bot. Ges. 9: 229-232.
- Pickering, S. U., and the Duke of Bedford. 1908. Woburn Exp. Fruit Farm, Rept. 8.
- , 1910. Woburn Exp. Fruit Farm, Rept. 11.
- Plester, W. 1912. Kohlensäureassimilation und Atmung bei Varietäten derselben Art, die sich durch ihre Blattfärbung unterscheiden. Beitr. Biol. Pflanz. 11: 249-304.
- Rosé, E. 1913. Énergie assimilatrice chez les plantes cultivées sous différents éclaircements. Ann. Sci. Nat. Bot. IX, 17: 1-110.

- Rumm, C.** 1893. Ueber die Wirkung der Kupferpräparate bei Bekämpfung der sogenannten Blattfallkrankheit der Weinrebe. *Ber. Deutsch. Bot. Ges.* **11**: 79-93.
- Schander, R.** 1904. Über die physiologische Wirkung der Kupfervitriolkalkbrühe. *Landw. Jahrb.* **33**: 517-584.
- Schertz.** 1919. Reported by W. Crocker. *Bot. Gaz.* **67**: 447.
- Schimper, A. F. W.** 1903. *Plant geography upon a physiological basis* (Eng. transl.). Oxford.
- Spoehr, H. A., and Long, Frances.** 1919. The interrelation of photosynthesis and respiration. *Yearbook Carnegie Inst. Wash.* **18**: 78-80.
- True, R. H., and Gies, W. J.** 1903. On the physiological action of some of the heavy metals in mixed solutions. *Bull. Torrey Bot. Club* **30**: 390-402.
- Willstätter, R., and Stoll, A.** 1913. *Untersuchungen über Chlorophyll, Methoden und Ergebnisse.* Berlin.
- . 1915. Untersuchungen über die Assimilation der Kohlensäure. *Ber. Deutsch. Chem. Ges.* **48**: 1540-1563.